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Epidemiological aspects of cutaneous leishmaniasis in the Iguazú falls area of Argentina

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ABSTRACT

Over the last three decades the incidence of American cutaneous leishmaniasis (ACL) has increased sharply in Argentina and throughout the world. In the Iguazú Falls area, on the border between Brazil and Paraguay, the incidence of human ACL has risen since 2004. Most of the 36 cases of human ACL reported until 2005 have involved males over 15 years old (75%) infected during deforestation to establish individual farms. Captures carried out in primary forest, periurban areas, and deforested land sites yielded 18,438 sand flies belonging to 13 species; the most prevalent species were *Lutzomyia (Nyssomyia) whitmani* (87.4%) and *Lutzomyia (Mygonemyia.) migonei* (7.6%). Cluster analysis was used to group traps according to species and abundance of sand flies. The group of traps located in recently deforested places, in pig and chicken dwellings of houses where ACL cases had been reported in the past, and at one house with an active ACL case, had the highest abundance of *Lu. whitmani* and *Lu. whitmani* + *Lu. migonei* as well as the highest ratio of *Lu. whitmani*/*Lu. migonei*. *Leishmania* sp. infections, both in *Lu. whitmani*, in *Lu. quinquefer*, and in smears from human cases were detected by DNA kinetoplast amplification using a generic PCR protocol. The risk of ACL outbreak in the Iguazú Falls area is still associated with economic and leisure activities in primary-secondary forest, including deforestation, rural settlements, fishing, hunting, and ecotourism. In addition, the risk of periurban transmission seems likely, and this is discussed within the framework of surveillance and prevention strategies.

1. Introduction

The incidence of American cutaneous leishmaniasis (ACL) has increased steadily since the early 1980s, and it is spreading to new epidemiological scenarios. Man-made "ecological chaos" – from war, migration, unorganized peri-urbanization, deforestation, local climate change, HIV pandemics, and adaptation to the peridomiciliary environment of permissive vectors – has been proposed as the main driver of this disease emergence (Desjeux, 2001; Campbell-Lendrum et al., 2001; Ashford, 2007; Shaw, 2007). Thus, any focus in a new eco-epidemiological scenario near populated cities should be characterized to assess epidemic trends.

In Argentina, the incidence of ACL due to *Leishmania braziliensis* has increased in peridomestic environments during the last two decades, and *Lutzomyia neivai* has been implicated as the main vector (Córdoba-Lanús et al., 2006; Salomón et al., 2006). The province of Misiones is located in northeastern Argentina in the subtropical area of Amazonian domain, and it is almost completely surrounded by rivers. Coniferous woods have replaced most of the native trees in the forest in the Iguazú Falls area (Iguazú National Park, Urugua-í Provincial Reserve) on the Border between Argentina, Brazil and Paraguay. Close to this area, 129 cases of ACL were reported in 1998 from a peri-urban neighborhood of Puerto Esperanza Village (incidence 4.2%), although only nine cases of ACL had been reported for the whole province from 1977 to 1997 (Salomón et al., 2001, 2002). Another 20 cases were reported during 2003–2004, clustered in time and space among deforestation-related workers with activities around the Urugua-í artificial lake area (incidence 5%), 15 km away from the previous focus (Salomón et al., 2006). Puerto Esper-

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anza outbreak was associated with peridomestic transmission and *Lu. neivai* abundance, whereas Urugua-i cases were associated with activities at the edge of the forest and *Lutzomyia whitmani* presence.

Since 2004, the rate of reported ACL cases from Puerto Iguazú has been increasing. Puerto Iguazú City is located at the trinational border and has a resident population of 31,000, and it has many more non-residents due to commercial and tourist activities. More than a million tourists from all over the world visit Iguazú Falls and the National Park every year. Therefore, in this ACL transmission scenario, large populations may be at risk, and there is also a lack of knowledge of the eco-epidemiology of emergent foci.

The aim of this work is to describe this new scenario for ACL transmission in the southernmost area of the Amazon region from entomological and parasitological perspectives. The results will contribute to a better understanding of the epidemic shift of ACL in America from sylvatic to periurban environments, thereby providing the basis for appropriate control measures.

2. Materials and methods

2.1. Study area and human cases

The study area was the department of the northern area of Iguazú in the province of Misiones, Argentina (25° 36′ S, 54° 35′ W). The area includes Puerto Iguazú City, the National Park, and the Provincial Natural Reserve. The city of Puerto Iguazú is surrounded by the border with Paraguay and Brazil on the shore of the Paraná-Iguazú rivers at altitudes ranging of 140–240 m above sea level. The phytogeographic area is classified as Paranaense Forest: a subtropical humid forest from the Amazonian domain (Cabrera, 1971). The area of primary-secondary forest known as "2000 hectáreas" (2K), which lies south of the city, west of Iguazú Falls National Park, and north of the Urugua-í Provincial Park, has experienced the following human interventions: (a) since 1950, wood extraction by the army; (b) in 2001–2002, marginal deforestation and human settlement, mainly in a narrow area bordering the road; (c) since 2003 and peaking in 2004, intense deforestation in patches of the remaining area by newly settled, individual farmers (Fig. 1).

The clinical files of the ACL cases diagnosed during 2004–2005 at the SAMIC Referential Hospital of Iguazú city were checked to compute statistics on sex, age, probable site of infection, and probable date of infection.

2.2. Sand fly collections

Adult sand flies were captured with minilight traps (Sudia and Chamberlain, 1962) operated from 17:00 to 09:00 h. The traps were placed at a height of 1.5 m and were shadowed by the canopy. Collections were performed at each site three times between 26 October and 12 November 2005. The traps were located at 14 different capture stations (Fig. 1), and 29 capture sites including different habitats of the domiciliary capture stations (Table 1). The capture stations H1 and H3 were houses where individuals that had ACL in July and August 2005 respectively lived; H2 was a house where an active ACL case was reported. All captured sand flies were stored dry (except those used for molecular research on parasites, which were kept at −20 °C) and identified using the keys of Young and Duncan (1994), with modifications by Marcondes (1996).

2.3. Sand fly abundance and diversity analysis

Cluster analyses were performed to differentiate sites according to the species composition and abundance. The matrix included the 3-day cumulative abundance of 10 species of phlebotomine caught at the 26 sites with sand flies. The three species of *Brumtomyia* were combined as *Brumptomyia* spp. because the females were morphologically indistinguishable; the F1, H7, and P2 sites with no sand flies were excluded. The Bray-Curtis quantitative dissimilarity coefficient was computed (relative distance from 0 for a pair of sites with identical taxonomic composition and abundance to 1) (Bray and Curtis, 1957), the group average was used as a group linkage method (Infostat, 2007^{TM}), and the 0.5 distance was used as a criterion for building the cluster. One-way ANOVA was used to test differences between groups for three variables: (a) *Lu. whitmani* + *Lu. migonei* abundance (*Lw* + *Lmg*), (b) *Lu. whitmani*/*Lu. migonei* rate (*Lw*/*Lmg*), and (c) *Lu. whitmani* abundance (*Lw*). Data were transformed by *ln* prior to statistical analysis in order to comply with the assumptions of normality and homocedasticity. When significant differences were found, means were compared using an *a posteriori* Tukey test (significance at $p \leq 0.05$) (Zar, 1996). In addition, a diversity index ($D = 1 - \Sigma pi^2$) was computed for all sites, *pi* is the proportion of sand flies of the species *i* in each capture site *p* (Krebs, 1978).

2.4. Molecular detection of parasites

2.4.1. Sand fly sample

From the original capture of sand flies, a random sample of 138 females and 10 males as a negative control for PCR was selected for molecular detection of the *Leishmania* sp. parasite. Females were pooled according the species and capture site in groups varying from 10 (*Lu. whitmani* × 12 pools), to 8 (*Lu. pessoai* × 1), 5 (*Lu quinquefer* × 1), 5 (*Lu. migonei* × 1) individuals, besides the pool of 10 males of *Lu. whitmani*. DNA extraction was performed by a protocol involving proteinase K digestion, phenol purification, and ethanol precipitation (Pita-Pereira et al., 2005). DNA was frozen at −20 °C until use.

2.4.2. Clinical samples

Samples were obtained from three patients with clinical symptoms; positive smears were taken as dermal scrapings from the border of the ulcers with a sterile wooden toothpick. The samples were taken at the SAMIC Hospital, Puerto Iguazú on 8–21 August 2005; the ulcers selected for scraping had been developing for 3–5 months. The probable site of transmission was used to select entomological trapping sites within 2K. Informed consent was obtained from all participants, and all procedures were in accordance with the Helsinki Declaration. The samples were stored in 200 μl of TE buffer. DNA extraction was performed by the boiling protocol, which involves heating the samples for 10 min then freezing them at −20 °C until use (Belli et al., 1998).

2.4.3. Analysis by polymerase chain reaction (PCR)

The PCR was performed as described by Belli et al. (1998). The genus–specific primers (13A, 13B) amplified a 120 bp product which target in the conserved block (CSB) of the DNA kinetoplast present in most species of the *Leishmania* genus. *L. braziliensis* HOM/BR75M2903 and the *L. amazonensis* IFLA/BR67/PH8 strains were employed as PCR controls. These strains were provided by the National Institute of Parasitology "Dr. Mario Fatala Chabén" (Buenos Aires, Argentina).

3. Results

During 2004–2005, 36 cases of ACL were reported by the referral Hospital of Puerto Iguazú; 27 (75%) were males, and 9 (25%) were younger than 15 years old. The geographic site where transmission was self-reported to occur was the 2K area for 31 (86%) cases, and another 4 (11%) were due to work related to primary forest and leisure activities, including the National Park,